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ORIGINAL RESEARCH

Association of vitamin D deficiency and hyperparathyroidism with anemia: a cross-sectional study

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Abstract: Vitamin D deficiency and anemia are common in the Middle East, and vitamin D deficiency and hyperparathyroidism have been reported to be associated with an increased prevalence of anemia. In this study, the hypothesis that vitamin D deficiency and hyperparathyroidism may be associated with anemia in a Bahraini population was tested. Association of hyperparathyroidism and vitamin D levels (deficiency and insufficiency) with anemia was investigated in 421 Bahrainis (213 males and 208 females). In females, the prevalence of anemia was significantly associated with vitamin D deficiency independent of parathyroid hormone levels (odds ratio: 2.9; 95% confidence interval: 2.3–10.5; $P = 0.001$). In females, the prevalence of anemia appeared to be significantly associated with hyperparathyroidism (odds ratio: 2.1; 95% confidence interval: 1.2–3.7; $P = 0.01$); however, this significant association disappeared when adjusted for vitamin D deficiency (odds ratio: 1.6; 95% confidence interval: 0.75–6.5; $P = 0.154$). Results from this study suggest that vitamin D deficiency is independently associated with anemia in females but not males. Further studies to determine whether vitamin D supplementation could be used to treat anemia are warranted.

Keywords: vitamin D, deficiency, hyperparathyroidism, anemia

Introduction

The prevalence of hypovitaminosis D has been reported to be high in various regions around the Middle East. Despite ample sunshine throughout the year, there are a large number of studies in the past decade suggesting that one-third of individuals living in Sub-Saharan Africa and the Middle East have serum 25-hydroxyvitamin D (25[OH]D) levels <25 nmol/L.[1] In addition, to the well-documented role of vitamin D in the regulation of bone and mineral metabolism, vitamin D may have an effect on erythropoiesis including cellular proliferation and differentiation and induction of erythroid progenitors in bone marrow.[2] Higher levels of 1,25-dihydroxyvitamin D (the active form of vitamin D) in bone marrow compared to plasma have been reported.[3] Hyperparathyroidism has also been suggested to induce bone marrow fibrosis and suppress erythropoiesis – especially in subjects with secondary hyperparathyroidism, and vitamin D treatment of subjects with hyperparathyroidism has been shown to improve the response to erythropoietin therapy.[4,5,6] In addition, low 25(OH)D levels are reported to be associated with low hemoglobin concentrations in individuals with normal renal function and also patients with chronic renal disease.[7,8,9]

Most published epidemiological studies and clinical trials have focused on the possible protective effect of vitamin D against cancer[10,11] and risk of cardiovascular disease.[12,13] Information regarding the association of vitamin D deficiency and

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hyperparathyroidism with anemia is limited especially in Middle Eastern populations.

The Kingdom of Bahrain is a small and sunny island that presents an opportunity to investigate the risk factors associated with hypovitaminosis D. In this study, the hypothesis that vitamin D insufficiency, deficiency, and hyperparathyroidism may be associated with anemia in Bahraini males and females was tested.

Subjects and methods

This study was conducted between October 2010 and October 2011 in healthy volunteers attending the blood bank center in Bahrain Defense Force hospital, the second largest hospital in the Kingdom of Bahrain. Subjects were asked about their willingness to participate in the study and eligibility. All subjects completed a questionnaire on age, gender, pregnancy, and history of any chronic diseases such as diabetes mellitus, hypertension, obesity, osteoporosis, osteomalacia, liver disease, renal disease, anemia, hypo- or hyperparathyroidism, vitamin and mineral deficiency, and steroid therapy. They were also asked about medications that could interfere with vitamin D metabolism, vitamin therapy, and whether they were taking vitamin D and calcium supplements during a face-to-face interview. Of the 700 who attended the blood bank and were asked to participate in the study, 66 were non-Bahrainis; of the remaining 634, 550 agreed to participate (a response rate of 87%). One-hundred and twenty-nine were subsequently excluded because of obesity (>25 kg/m^2), history of liver, renal, gestational, or endocrine disorder, medications that influence bone metabolism and current vitamin D and calcium intake, history of iron, folate, and vitamin B12 deficiencies, aplastic anemia, hemolytic anemia, sickle cell anemia, and thalassemia.

Therefore, 208 women (aged 18–58 years) and 213 men (aged 18–60 years) were included in the final study. All participants gave written consent after being fully informed of the study objectives and procedures and their right to withdraw from the study. This study complied with the principles of the Declaration of Helsinki (2008). The research protocol was approved by the Research and Ethics Committees of the College of Medicine and Medical Sciences, Arabian Gulf University, and Research and Ethic Committee of Bahrain Defense Force hospital.

Fasting serum calcium and phosphorus, creatinine, and bone specific alkaline phosphatase were analyzed immediately using the Cobas® 6000 analyzer system (Roche Diagnostics, Basel, Switzerland). Hemoglobin levels were determined by Cell-Dyn® 3700 (Abbott Laboratories, Abbott Park, IL, USA). Fasting plasma intact parathyroid hormone (PTH) was determined using commercially available enzyme-linked immunosorbent assay kits (Creative Diagnostics, Shirley, NY, USA). The intra-assay and inter-assay coefficients of variation for determination of intact PTH in plasma were less than 4.5% and 7.1% for low control and 3.2% and 5.2% for high control, respectively. Fasting serum total 25(OH)D (25[OH]D_3 and 25[OH]D_2) concentrations were determined by ultra performance liquid chromatography tandem mass spectrometry (Waters Limited, Elstree, UK) using commercially available kits (Chromsystems Instruments and Chemicals GmbH, Grafelfing, Germany). The intra-assay and inter-assay coefficients of variation for determination of 25(OH)D_3 in serum were less than 2.7% and 3.9% for low control and 4.2% and 4.0% for high control, respectively. The intra-assay and inter-assay coefficients of variation for determination of 25(OH)D_2 in serum were less than 3.9% and 5.7% for low control and less than 4.3% and 4.7% for high control, respectively. Vitamin D deficiency was defined as serum total 25(OH)D concentration <30 nmol/L, insufficiency at levels between 30–50 nmol/L, and optimal at levels ≥ 50 nmol/L.[14] Anemia was defined according to World Health Organization criteria with hemoglobin <13 g/dL in men and <12 g/dL in women. Hyperparathyroidism was defined as plasma PTH levels >65.0 pg/mL and hypocalcemia was defined as serum calcium <2.1 nmol/L. These cutoff values are based on the reference range determination of these parameters determined in the authors' clinical laboratory in the Kingdom of Bahrain.

Statistical analysis

The 2.5th, 5th, 50th, 95th, and 97.5th percentiles were determined to observe the distributions of biometric and biochemical parameters in the participants of this study. The normality of serum total 25(OH)D and plasma PTH distributions was assessed using the Kolmogorov–Smirnov test. As they were negatively skewed, they were logarithmically transformed to reduce kurtosis before geometric means were calculated. Thus, logarithmic transformations were used in further statistical analysis.

Student's *t*-test was used to compare the biometric and biochemical parameters between males and females. Analysis of variance and Scheffe's post hoc test were used to compare the hemoglobin and other biometric and biochemical parameters between participants with optimal, insufficiency, and deficiency of vitamin D. Pearson's correlation coefficients were used to examine the correlation between hemoglobin levels with serum total 25(OH)D, PTH, and other variables.

Stepwise multiple regression analysis was used to determine the predictors of blood hemoglobin using age, alkaline phosphatase, total 25(OH)D, creatinine, phosphorus, calcium, and PTH as independent variables.

Univariate and multivariate logistic regression analysis were used to determine the association of anemia with vitamin D insufficiency and deficiency adjusted for PTH as well as the association of anemia with hyperparathyroidism adjusted for total 25(OH)D. All statistical inferences were made based on a two-sided significance level of $P < 0.05$ and were performed using IBM® SPSS® Statistics version 19.0 (IBM Corporation, Armonk, NY, USA).

Results

The distributions of biochemical and biometric parameters in males and females are shown in Table 1. Serum total 25(OH)D was significantly higher in males than females, whereas plasma PTH was significantly higher in females than males.

In Table 2, the biochemical and biometric characteristics of participants of this study according to the vitamin D status are shown for females and males. In females, analysis of variance shows that age, calcium, and hemoglobin were significantly decreased and PTH was significantly increased in vitamin deficient individuals than in those with optimal and insufficient vitamin D levels. Scheffe's post hoc test for multiple comparison analysis shows that among all the parameters, hemoglobin was significantly lower in females with vitamin D deficiency ($P \leq 0.001$) and insufficiency ($P \leq 0.001$) compared with those with optimal levels of vitamin D. However, PTH was significantly higher in females with vitamin D deficiency compared with those with optimal levels of vitamin D ($P \leq 0.03$). In males, there were no significant differences between vitamin D groups in any parameters listed in Table 2.

Further statistical analysis indicates that in females hemoglobin was significantly and negatively correlated with PTH ($r = -0.254$, $P = 0.0001$) and significantly and positively correlated with total 25(OH)D ($r = 0.338$, $P = 0.001$). Stepwise multiple regression analysis showed that in females the predictors of hemoglobin were total 25(OH)D ($\beta = 0.319$, $P \leq 0.001$) and PTH ($\beta = -0.227$, $P = 0.01$), contributing to 41.8% and 18.9% variance in hemoglobin, respectively.

In Table 3, univariate and multivariate logistic regression analysis shows that in females there was a significant 2.1-fold increased risk of anemia with hyperparathyroidism. However, when adjusted for vitamin D, this association disappeared and was no longer significant. In contrast, there was a significant 3.3-fold increased risk of anemia with vitamin D deficiency and this remained significant (2.9-fold) after adjustment for hyperparathyroidism. In males, there was no significant association between anemia and hyperparathyroidism or vitamin D deficiency.

Discussion

This is the first population-based study to provide evidence that vitamin D deficiency is associated with anemia in healthy female Bahrainis. The association of vitamin D deficiency with an increased risk of anemia has been reported in some cross-sectional studies and also in patients with chronic kidney disease.[15,16] The exact mechanism of association of vitamin D deficiency with anemia is still not known. It is suggested that vitamin D deficiency could lead to increased risk of reticulocytosis and iron deficiency anemia.[8] In bone marrow, there are enormous vitamin D receptors and vitamin D is reported to stimulate erythroid precursors. High local concentrations of 1,25 di hydroxyvitamin D in hematopoietic tissues is suggested to activate erythroid precursor cells in a paracrine fashion.[17,18] In addition, high

Table 1 Distribution of biometric and biochemical parameters in males and females

Characteristics	Females						Males					
	Mean (SD)	Percentiles					Mean (SD)	Percentiles				
		2.5	5	50	95	97.5		2.5	5	50	95	97.5
Age (year)	35.7 ± 11.6	20.0	20.0	33.0	55.0	58.0	34.4 ± 9.8	18.0	20.0	33.0	51.0	54.0
Phosphorus (mmol/L)	1.3 ± 0.31	0.88	0.92	1.20	1.9	2.2	1.1 ± 0.23	0.69	0.76	1.1	1.5	1.6
Calcium (mmol/L)	2.2 ± 0.18	1.2	1.9	2.3	2.5	2.5	2.4 ± 0.15	2.0	2.1	2.3	2.6	2.7
PTH (pg/mL)†	69.4 ± 44.4#	16.7	18.1	53.1	284.9	315.8	32.7 ± 28.1	5.8	6.7	26.9	371.0	681.4
Hemoglobin (mg/dL)	12.2 ± 1.4	9.7	9.8	11.9	15.1	15.3	14.5 ± 1.7	10.7	11.5	15.0	16.6	16.8
Alkaline phosphatase (U/L)	73.4 ± 22.9	34.9	46.0	69.0	123.1	137.3	79.8 ± 29.5	41.8	47.0	74.0	132.9	165.2
25(OH)D (nmol/L)†	22.4 ± 9.6	4.8	7.1	23.7	65.9	89.9	34.1 ± 25.1*	10.2	13.5	35.9	69.4	78.9
Serum creatinine (μmol/L)	63.2 ± 20.1	35.2	37.4	42.1	61.3	66.5	84.5 ± 27.4*	46.8	49.7	61.1	81.3	86.9

Notes: *Significantly higher in males than females; #significantly higher in females than males; †geometric mean.
Abbreviations: 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; SD, standard deviation.

Table 2 Biochemical and biometric characteristics of the males and females according to the vitamin D status

Characteristics	Females				Males			
	Optimal (n = 21)	Insufficiency (n = 54)	Deficiency (n = 133)	ANOVA *P*-value	Optimal (n = 43)	Insufficiency (n = 106)	Deficiency (n = 64)	ANOVA *P*-value
Age (years)	37.5 ± 13.9	36.7 ± 10.9	33.1 ± 10.1	0.04	34.1 ± 9.0	34.3 ± 10.1	34.4 ± 9.9	0.991
Phosphorus (mmol/L)	1.4 ± 0.40	1.2 ± 0.29	1.3 ± 0.23	0.148	1.1 ± 0.21	1.1 ± 0.29	1.1 ± 0.21	0.958
Calcium (mmol/L)	2.4 ± 0.15	2.2 ± 0.16	2.1 ± 0.22	0.04	2.4 ± 0.16	2.4 ± 0.15	2.3 ± 0.13	0.443
PTH (pg/mL)	38.6 ± 37.1	45.9 ± 42.8	89.9 ± 66.8[#]	0.03	38.5 ± 35.2	30.4 ± 27.2	39.9 ± 29.6	0.117
Hemoglobin (mg/dL)	13.0 ± 1.4	12.9 ± 1.3*	10.6 ± 1.6*[†]	<0.001	14.6 ± 1.7	14.5 ± 1.6	14.3 ± 1.7	0.577
Alkaline phosphatase (U/L)	77.1 ± 30.1	69.9 ± 17.3	73.2 ± 21.3	0.326	78.7 ± 30.4	79.1 ± 24.6	81.5 ± 33.5	0.835
Serum creatinine (μmol/L)	59.8 ± 15.6	68.5 ± 25.5	61.3 ± 21.2	0.425	89.5 ± 32.4	82.4 ± 25.8	81.6 ± 24.1	0.694

Notes: Total 25-hydroxyvitamin D: optimal >50 nmol/L; insufficiency 30–50 nmol/L; deficiency <30 nmol/L. All results are expressed as mean ± standard deviation. *Significantly lower than those with an optimal level of total 25-hydroxyvitamin D ($P \leq 0.001$, Scheffe's post hoc test); [†]significantly lower than those with optimal and insufficient levels of total 25-hydroxyvitamin D ($P \leq 0.001$, Scheffe's post hoc test); [#]significantly higher than those with an optimal level of total 25-hydroxyvitamin D ($P \leq 0.03$, Scheffe's post hoc test).
Abbreviations: ANOVA, analysis of variance; PTH, parathyroid hormone.

doses of calcitriol – the active form of vitamin D – has been widely used to increase hemoglobin levels and reticulocyte count in hematological disorders.[19,20] In vivo and in vitro studies have also demonstrated that calcitriol reduces cytokine production leading to the reduction of inflammatory milieu and anemia.[3]

An inverse relationship between plasma 25(OH)D and PTH levels in females was also observed in this study. Although this relationship was not statistically significant following multivariate regression analysis, it is consistent with a large number of studies in different populations and suggests that hypovitaminosis D could lead to secondary hyperparathyroidism, which is harmful to bone health.[21,22] It is now well established that vitamin D causes the suppression of PTH synthesis by increasing plasma calcium and by acting on parathyroid cells.[23,24] In addition, there was a positive correlation between 25(OH)D and hemoglobin and a negative correlation between hemoglobin and PTH. Moreover, stepwise multiple regression analysis showed that total 25(OH)D and PTH were the main and independent predictors of hemoglobin, but total 25(OH)D was a better predictor than PTH (41.90% versus 18.0% variance in hemoglobin). In addition, although the results from logistic regression analysis shows that there was a significant association of vitamin D deficiency and hyperparathyroidism with anemia, this significant association of hyperparathyroidism with anemia disappeared when adjusted for 25(OH)D. This study demonstrates that vitamin D-deficient females are prone to hyperparathyroidism and anemia.

Over the past 30 years, in vitro and in vivo studies suggest that PTH plays a significant inhibitory role in erythropoiesis and survival. Secondary hyperparathyroidism is known to induce bone marrow fibrosis, impair erythropoiesis, and inhibit the endogenous production of erythropoietin. PTH is

Table 3 Univariate and multivariate logistic regression analysis for association of hyperparathyroidism and vitamin D deficiency and insufficiency with low hemoglobin in females and males

	Females				Males			
	Anemic (<12 mg/dL) (n = 106)	Non-anemic (>12 mg/dL) (n = 102)	OR (95% CI)	*P*-value	Anemic (<13 mg/dL) (n = 51)	Non-anemic (>13 mg/dL) (n = 162)	OR (95% CI)	*P*-value
PTH (pg/mL)								
<65	48	64	1.0		38	127	1.00	
>65	58	38	2.1 (1.2–3.5)	0.01	13	35	1.2 (0.59–2.6)	0.568
			1.6 (0.75–6.5)[†]	0.154			0.94 (0.6–2.9)[†]	0.324
Total 25(OH)D								
>50	7	14	1.0		9	34	1.0	
30–50	16	38	0.8 (0.28–2.4)	0.754	24	82	1.1 (0.47–2.6)	0.819
<30	83	50	3.3 (1.3–8.8)	0.02	18	46	1.5 (0.59–3.7)	0.402
			2.9 (2.3–10.5)[‡]	0.001			1.6 (0.97–3.8)[‡]	0.312

Notes: [†]Adjusted for vitamin D; [‡]adjusted for PTH.
Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; OR, odds ratio; PTH, parathyroid hormone.

reported to be a resistance factor in recombinant human erythropoietin therapy.[5]

A marked gender difference in vitamin D status has been reported by the authors' group and the prevalence of vitamin D deficiency is significantly higher in females than in males in the Kingdom of Bahrain.[25] The lower vitamin D status in females compared to males observed in the present study could be explained by females spending more time indoors than males and/or the type of clothing that females wear and sun protection and sun avoidance attitudes seen in Bahraini women.[26]

The association of vitamin D deficiency with anemia observed in this study is consistent with some recent studies in different populations. Perlstein et al reported that vitamin D deficiency is associated with specific subtypes of anemia in the elderly, especially in those with anemia of inflammation.[15] Sim et al reported an increased prevalence of vitamin D deficiency associated with anemia in a cross-sectional study.[8] In addition, observational studies have shown that African Americans have lower circulating levels of 25(OH)D and are more likely to be vitamin D deficient than other ethnic groups, and it is suggested that increased prevalence of anemia in non-Hispanic blacks is correlated with vitamin D deficiency.[27]

There are some limitations in this study including the lack of data of other confounding factors related to erythropoiesis, lack of 1,25 dihydroxyvitamin D measurement, lack of information about other causes of anemia including iron, B12, and folate deficiency as well as sickle cell and thalassemia carriers among the participants (although individuals with history of anemia were excluded from this study).

Although this study demonstrates an association of vitamin D deficiency with anemia, a causal relationship cannot be established and further studies are warranted to investigate the relationship of vitamin D deficiency and hyperparathyroidism with causes of anemia in anemic patients in the Kingdom of Bahrain.

Conclusion

The results of this study indicate that vitamin D deficiency is associated with anemia in healthy Bahraini females. These findings could have potentially broad public health implications given the high prevalence of vitamin D deficiency in Bahrainis.[31] These findings suggest that further investigations are warranted – particularly those examining specific erythropoiesis and inflammatory pathways or host genetic changes that contribute to the pathogenesis of anemia – before vitamin D deficiency can be implicated in the causal development of anemia. Ultimately, if these results can be replicated by others and extended, they could lead to randomized clinical trials to evaluate vitamin D supplementation as therapy for patients with anemia.

Disclosure

The authors report no conflicts of interest in this work.

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